About Joseph W. Kellum

HOMETOWN
Baxter Springs, Kan.

MAJOR
Neurobiology

ACADEMIC LEVEL
Senior

RESEARCH MENTOR
James A. Orr
Professor of Molecular Biosciences

Q&A

How did you become involved in doing research?
I met Dr. Orr in an advising meeting my freshman year. He was very helpful and encouraging, so when it came time for me to search for a lab to obtain research experience, I sought Dr. Orr first. I read about his research projects on the KU website, and then sent him an email expressing my interest. We set up a meeting to discuss the lab and the possible roles I might be able to play. The next semester, I began assisting older students in their research projects, and eventually was able to complete a project of my own.

How is the research process different from what you expected?
I originally became involved in research because I knew it was essential for admission to a good medical school. At this point, I viewed it as another pre-med requirement; however, my opinions have since changed dramatically. I found research to be an incredibly exciting field. The process of asking questions, designing procedures to answer said questions, and revealing previously unknown knowledge is very fulfilling. Now, just over three years after my initial exposure to undergraduate research, I firmly believe that conducting biomedical research will be an essential component of my career as a physician.

What is your favorite part of doing research?
My favorite part of research is seeking out questions that are scientifically and clinically significant, and then brainstorming to come up with possible explanations for unexplained phenomena. This initial portion of the research process—before experimental design and execution—is, to me, the most exciting and intriguing part of research.

Inhibition of L-type and cyclic nucleotide-gated calcium channels demonstrates synergistic mechanisms for prolonging vascular contractions induced by a mimetic of thromboxane A_2

Joseph W. Kellum

INTRODUCTION

Thromboxane A_2 (TxA_2) is a metabolite of arachadonic acid—an unsaturated membrane phospholipid. TxA_2 is released by platelets during tissue trauma and inflammatory events (1). This prostanoid signaling molecule is responsible for further platelet activation and aggregation, as well as constriction of smooth muscle comprising the walls of bronchioles and blood vessels (1). The current study examines the effects of TxA_2 on vascular smooth muscle.

It has been shown that TxA_2 evokes vasoconstrictive responses that persist for a longer duration than a number of other vasoactive molecules (e.g. phenylephrine, KCl and others) (2). This observation is of clinical importance, due to the fact that TxA_2 vascular contractions elicited *in vivo* have the potential to decrease blood supply to tissues for prolonged periods of time. Such circumstances could be life-threatening in the case of TxA_2 release during acute, traumatic events in which decreased blood flow leads to reductions in nutrient delivery and eventual cell death, such as myocardial infarction or stroke (3). In these situations, TxA_2 produced in response to tissue trauma would promote vasoconstriction, which would trigger a positive feedback cycle resulting in greater decreases in blood supply. Strong correlations

have also been observed between increased levels of thromboxane B_2 (a metabolite of TxA_2) and patients suffering from angina pectoris, which could be considered a chronic manifestation of the negative effects of TxA_2-induced vasoconstriction (4).

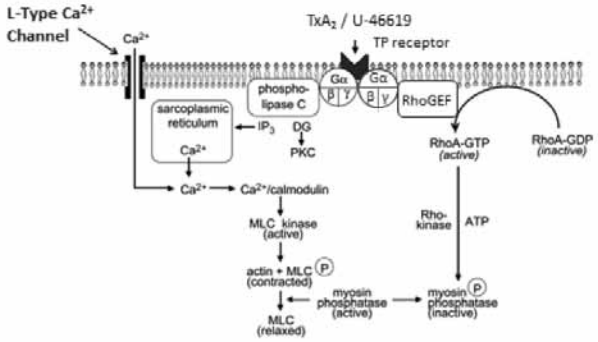
The primary purpose of this investigation was to gain a better understanding of the molecular mechanisms responsible for these characteristically prolonged vascular contractions elicited in vessels exposed to TxA_2. Unfortunately, TxA_2 is an unstable molecule, with a half-life of ~30 seconds in aqueous solution at 37°C (5). This instability makes use of the endogenous compound impractical in the laboratory. For this reason, a stable mimetic of TxA_2 (known as U-46619) was used to elicit the vascular contractions of interest throughout these experiments (6).

Both TxA_2 and U-46619 bind to the TP receptor to elicit cellular signaling cascades, ultimately leading to smooth muscle contraction. The TP receptor is a metabotropic, G protein-coupled receptor. Specifically, the activated G protein has a Gαq subunit which dissociates from the βγ subunit and diffuses into the cytosol to activate phospholipase C (PLC). Activated PLC cleaves membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2), producing inositol 1,4,5-triphosphate (IP_3) and diacyl glycerol (DG). IP_3 migrates to the sarcoplasmic reticulum (SR), where it binds to Ca^{2+} channels in the membrane of the SR. This permits the release of intracellular Ca^{2+} stores into the cytosol. Increases in cytosolic Ca^{2+} concentrations lead to activation of a number of Ca^{2+} dependent signal transduction cascades (1).

Diacyl glycerol (the portion of PIP_2 that remains in the membrane) then couples with Ca^{2+} in the cytosol to activate protein kinase C (PKC). Activated PKC phosphorylates L-type Ca^{2+} channels in the plasma membrane. Phosphorylation, coupled with cell membrane depolarization, leads to the opening of these L-type Ca^{2+} channels, which are both voltage-gated and ligand-gated. Opening of L-type Ca^{2+} channel permits influx of extracellular Ca^{2+}, which leads to greater increases in intracellular Ca^{2+} concentrations. Elsewhere in the cell, these increases in intracellular Ca^{2+} concentration lead to the formation of Ca^{2+}/calmodulin kinases, which activate myosin light chain kinase (MLCK) via phosphorylation. In turn, MLCK propagates the signal by phosphorylating myosin light chain (MLC). MLC then undergoes cross-bridge cycling with actin filaments, thus promoting contraction of the smooth muscle (1).

Concurrent with the mechanism for contraction discussed above, myosin phosphatase acts to dephosphorylate MLC. This promotes return of the smooth muscle tissue to a relaxed state. Myosin phosphatase is regulated by a signaling cascade known as the Rho-kinase pathway (1). This cascade also begins with a G protein coupled to the TP receptor (7). When activated, this Gα subunit activates a membrane-bound guanine-exchange factor, RhoGEF, which activates RhoA. RhoA then activates Rho-kinase, which phosphorylates myosin phosphatase. Phosphorylation of myosin phosphatase inactivates the enzyme. This shifts the contraction/relaxation equilibrium in favor of the contracted state, and serves as a mechanism for prolonging vascular contraction (1).

FIGURE 1.



Left is a flow chart of the signal transduction pathways for TxA_2-induced contraction in smooth muscle. Here, it can be seen that binding of the ligand (TxA_2 or U-46619) to the TP receptor activates two separate signal transduction pathways. One pathway leads to increases in intracellular Ca^{2+} concentrations, which promotes contraction. The other serves the purpose of maintaining established contractions. A more detailed description can be found in the text (1).

Unpublished data from this lab (8), as well as many published works over the past decade have demonstrated that inhibition of Rho-kinase does lead to significantly increased rates of relaxation in vessels initially contracted using U-46619 (9, 10, 11). Thus, it is apparent that the Rho-kinase pathway is necessary in establishing the prolonged nature of the U-46619-induced vascular contractions. However, this pathway has also been shown to be activated by a number of other vasoactive agents, none of which display these significantly prolonged contractions (10, 11). Phenylephrine (PE), for instance, binds to an α1 adrenergic receptor. This receptor has been demonstrated to be linked to both the Gαq protein responsible for establishing the U-46619 contraction, as well as the Rho-kinase pathway (10, 11). These similarities render the PE contraction a reliable control to which the U-46619 contraction can be compared. The difference, however, is that PE-induced vascular contractions relax within minutes following removal of PE from the bathing solution of isolated blood vessels, while U-46619-induced contractions persist for hours before returning to basal tension (6). Therefore, another unidentified mechanism must play a significant role in prolonging U-46619-induced contractions.

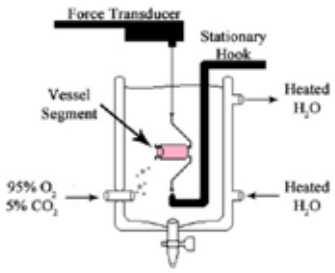
In work published from this lab, inhibition of L-type Ca^{2+} channels was examined in the context of two different types of contractions (6). Under normal circumstances, treatment of vascular smooth muscle with KCl leads to significant contractions due to depolarization of the cell membrane potential and opening of voltage-gated Ca^{2+} channels. When L-type Ca^{2+} channels were inhibited, KCl treatment elicited no measurable contractions. Inhibition of L-type Ca^{2+} channels was also effective in attenuating U-46619-induced contractions; however, the TxA_2 mimetic evoked measurable (though markedly reduced) contractions, even in the presence of these L-type Ca^{2+} channel inhibitors. The authors concluded that the U-46619 contraction mechanism likely utilizes more than one membrane channel for influx of extracellular Ca^{2+} (6). More recent work, published by Leung et al, introduced the idea that cyclic nucleotide-gated (CNG) Ca^{2+} channels may play a role in the U-46619-induced mechanism (12). This conclusion led to speculation that CNG Ca^{2+} channels might be the extra conduits for influx of extracellular Ca^{2+} referenced in the earlier work by Liu et al.

Therefore, the objective of this experiment was to inhibit both L-type (using an L-type channel inhibitor known as nifedipine) and CNG Ca^{2+} channels (using a CNG channel inhibitor known as L-cis-diltiazem) to test whether they played roles—either separately, or in combination—in prolonging the TxA_2/U-46619-induced contraction. It was hypothesized that inhibition of L-type and CNG Ca^{2+} channels would lead to significant increases in rates of relaxation in U-46619-treated vessels. We also predicted that inhibition of only one channel at a time would lead to moderate increases in the observed rate of relaxation, while simultaneous inhibition of both channels would lead to significantly greater increases in rates of relaxation.

MATERIALS AND METHODS

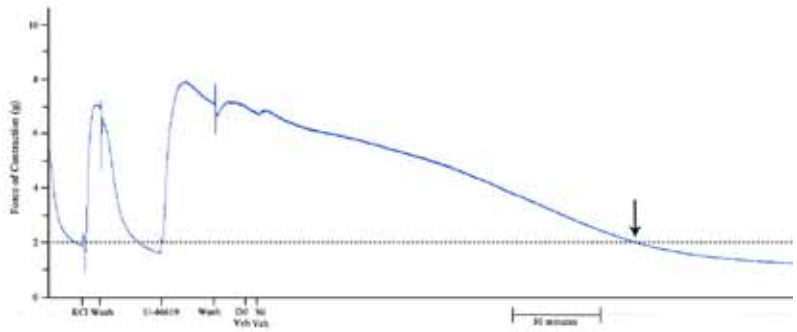
To test the effects of L-type and CNG Ca^{2+} channel inhibitors (nifedipine and L-cis-diltiazem, respectively) on U-46619-induced vascular contractions, segments of aorta were removed from euthanized New Zealand white rabbits (IACUC: AUS 42-02). Vessels were placed in a modified Krebs solution (6) and kept overnight at 40°C. All experiments were carried out the following day using a conventional isolated organ bath preparation. 95% O2/5% CO2 was bubbled through a physiological buffer (pH ~ 7.4), which was maintained at 37°C. The vessels were held in place by hooks, which applied tension in opposite directions. One hook was stationary, while the other relayed the contraction force created by the vessel to a force transducer (see figure 2 below). At the beginning of each experiment, a baseline tension of 2 grams of force was established to mimic the basal muscle tone that occurs in vivo.

FIGURE 2.



This figure is a schematic of the general organ bath setup utilized to measure tension elicited by the vessel throughout each experiment. As the vessel contracted, it applied tension to the force transducer shown at the top of the figure. The transducer relayed the magnitude of the force applied at a given time to a computer, which produced a graph like the one seen in figure 3, below.

FIGURE 3.



An example graph from a representative experiment is shown above. The first peak was caused by the KCl treatments, respectively. The second peak is representative of the U-46619-induced contraction. Notice how the peak caused by U-46619 plateaued at its maximum, whereas the KCl-induced peaks began to slope downwards even before the wash. Each treatment is marked along the x-axis, with Ni and Dil denoting nifedipine and L-cis-diltiazem, which are L-type and CNG Ca2+ channel inhibitors, respectively. The dotted line at 2 g of contraction signifies baseline tension, and the black arrow indicates the point at which the vessel returns to baseline tension following treatment with U-46619.

In each experiment, six individual segments of the aorta were placed in six separate organ baths. All six baths were subjected to the same general procedure to begin each experiment. Following a period of equilibration, the organ bath was filled with 60 mM KCl to induce maximal contraction, followed by a wash in which the KCl solution was drained from the bath and fresh buffer at pH 7.4 was added. The vessels were then allowed to relax to baseline tension. This sequence was then repeated once more. Following the second wash, all vessels were subjected to the addition of 5μM U-46619 to elicit contraction. Once the contraction reached its peak, as recorded by the force transducers on a computerized graph (see figure 3 above), the bath was washed for a third time. Following this third wash, the protocol varied, dependent upon whether inhibitors or vehicles were added to each bath. Figure 3 below has been included for the purpose of easily relating this protocol to the experiment conducted.

In the variable portion of the experiment, the vessels were treated with four different combinations of drugs and respective vehicles. The drugs used included nifedipine, which is an L-type Ca^{2+} channel inhibitor, at 200 μM (dissolved in 0.4% ethanol/99.6% water), as well as L-cis-diltiazem, which is a CNG Ca^{2+} channel inhibitor, at 140 μM (dissolved in 100% water). These dosages were obtained from previously published reports in which dose responses were carried out to determine effective doses of each drug (6, 12). Each drug was prepared by first preparing a concentrated stock solution, which also contained a greater concentration of ethanol in the case of nifedipine. This stock solution was then diluted upon addition of a small quantity (693 μL for nifedipine and 450 μL for L-cis-diltiazem) to 20 mL of physiological buffer in the organ bath during the experiment. Nifedipine and L-cis-diltiazem were purchased from Enzo Life Sciences, Farmingdale, NY. U-46619 was purchased from Cayman Chemical Co., Ann Arbor, MI.

The first treatment group consisted of vessels treated with both inhibitors, namely 200 μM nifedipine, followed by treatment of 140 μM L-cis-diltiazem. Following treatment, all vessels were allowed to relax to baseline tension and the relaxation time was recorded. The second group consisted of vessels treated with 200 μM nifedipine, followed by treatment with L-cis-diltiazem vehicle (100% water). The third group consisted of vessels treated with nifedipine vehicle (0.4% ethanol/99.6% water), followed by treatment with 140 μM L-cis-diltiazem. The fourth group consisted of vessels treated with nifedipine vehicle, followed by treatment with L-cis-diltiazem vehicle. This was the control set for this experiment.

As stated previously, vessel tension was monitored electronically throughout the duration of the experiment (see figure 4A – 4D). At the conclusion of each experiment, the graphs obtained were analyzed to yield quantitative data in the form of rates of relaxation for each vessel. This method of data analysis was chosen to normalize vessels that varied in the magnitude of the contraction. These rates were obtained by using the following formula:

$$\textit{Ave. Rate of Relaxation } (^{g}/_{min}) = \frac{\textit{Magnitude of contraction (in grams)-2.00 g}}{\textit{Time required to relax to baseline (in minutes)}}$$

RESULTS

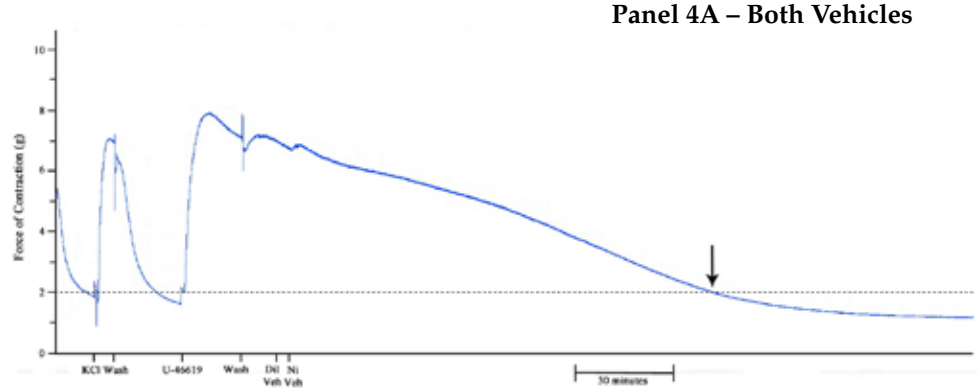
Computer-generated graphs produced during the course of an individual experiment are shown in figure 4 below. The y-axis of each graph displays vessel tension (grams). The x-axis displays time (minutes) throughout the duration of the experiment. Thus, the height of a line at any given point on the x-axis represents the force of contraction elicited by the vessel at that particular point in time.

Figures 4A – 4D display two different contractions: an initial KCl-induced contraction, followed by a U-46619-induced contraction. This comparison provides good visual representation of the prolonged nature of U-46619-induced contractions. While contractions elicited using KCl relax immediately following removal of the high KCl buffer (wash), the contractions produced by U-46619 treatment persist long after the drug has been removed from the organ bath.

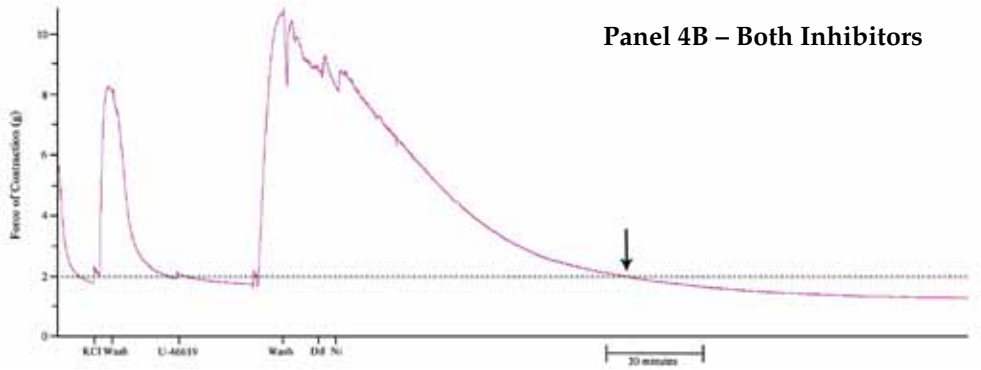
Figure 4 also facilitates qualitative comparison of the four different treatments given, and the rates of relaxation of each. The vessel represented in figure 4A was treated with the vehicles for each inhibitor. This served as the control group. It is obvious that the control group exhibited a decreased relaxation rate as compared to the groups treated with one or both inhibitors (shown in figures 4B – 4D). Figure 4B presents the responses of a vessel treated with both inhibitors, nifedipine and L-cis-diltiazem. The slope of this line is noticeably greater than that in figure 4A, but quite similar to the slopes in figures 4C and 4D, which were treated with L-cis-dilitiazem and nifedipine, respectively. This indicates that no significant differences were present between the relaxation rates of these three groups of inhibitor-treated vessels.

FIGURE 4.

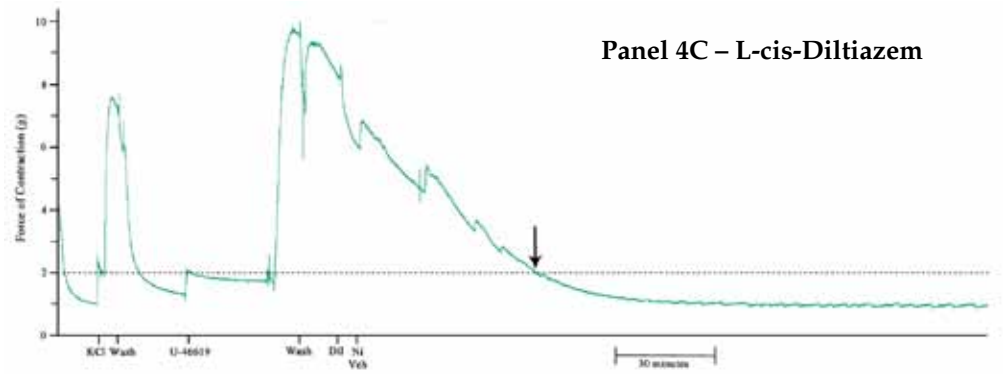
Panel 4A – Both Vehicles



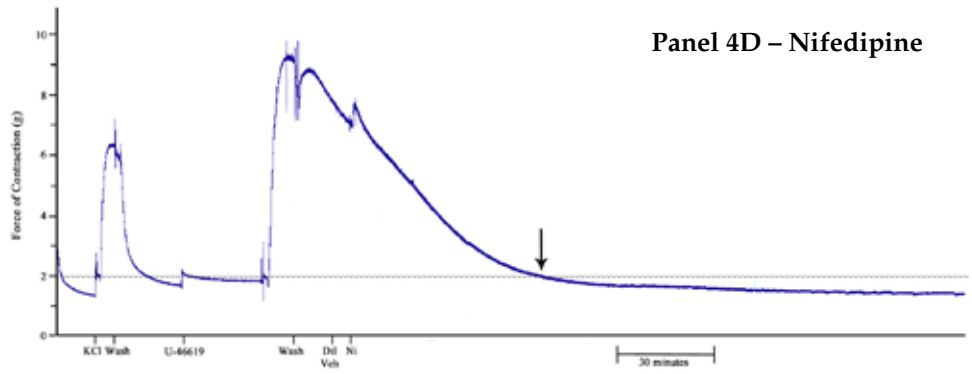
Panel 4B – Both Inhibitors



Panel 4C – L-cis-Diltiazem



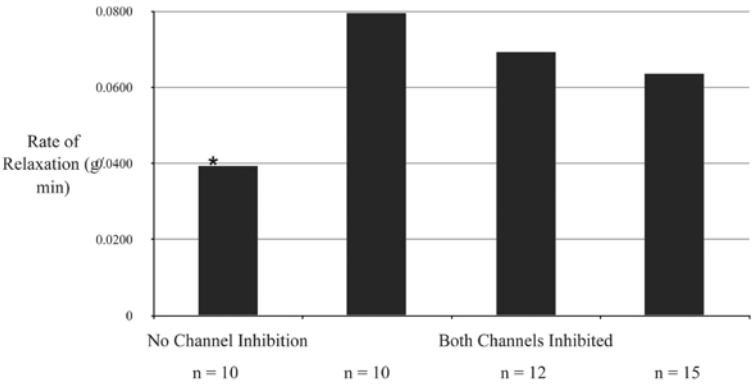
Panel 4D – Nifedipine



Data representing the contractions and relaxations of four vessels are shown. The panels are labeled according to which drugs they received prior to the relaxation phase. Notice how the vessels treated with various combinations of drugs reached baseline tension much more quickly than the vessel treated with vehicles only. On the x-axis, the treatment given at each point in time is indicated. Ni and Dil represent nifedipine and L-cis-diltiazem, respectively. Also, note that the arrows indicate the time at which each vessel returned to baseline tension after treatment with U-46619. Baseline tension (2 g) is indicated by the dotted horizontal line.

FIGURE 5.

Means Rates of Relaxation



The mean of the vehicle-treated group was shown to differ from the others in a statistically significant fashion using a one-way ANOVA (F-statistic = 7.61, 46 degrees of freedom, P < 0.001).

The figure is a graphical representation of the mean rates of relaxation (g/min) for each treatment group of interest, with bars representing the standard error of the mean. Statistical analysis of these data using an unstacked ANOVA indicated that the inhibitor-treated groups (L-cis-diltiazem, nifedipine, and both inhibitors) were statistically synonymous (F-statistic = 1.17; 31 degrees of freedom; P = 0.324). The same test, with the addition of the vehicle control treatment, indicated a strong statistical discrepancy between the four means (F-statistic = 7.61; 46 degrees of freedom; P < 0.001).

The mean rates of relaxation for all vessels within each treatment group are presented in figure 5. The control group (vehicle treatment only) had a significantly reduced mean rate of relaxation as compared to the three inhibitor-treated groups. This observation was tested using a one-way, unstacked ANOVA, which resulted in a test statistic of F = 7.61 with 46 degrees of freedom and P-value < 0.001, thus, supporting the initial hypothesis that inhibition of L-type and CNG Ca^{2+} channels will lead to increases in the rates of relaxation in U-46619-treated vessels.

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This graph also allows the mean rates of relaxation for the three inhibitor-treated groups to be compared. Small differences exist between these means, especially when comparing vessels treated with only L-cis-diltiazem to those treated with only nifedipine. However, statistical testing with a one-way, unstacked ANOVA in this instance failed to refute the null hypothesis for these means (F = 1.17; 31 degrees of freedom; P = 0.324). Therefore, these data indicate that no difference in relaxation rate is observed between vessels in which different Ca^{2+} channels are inhibited, as long as at least one channel is inhibited.

DISCUSSION

Role of L-type and CNG Ca^{2+} channels in prolonging U-46619-induced vascular contractions

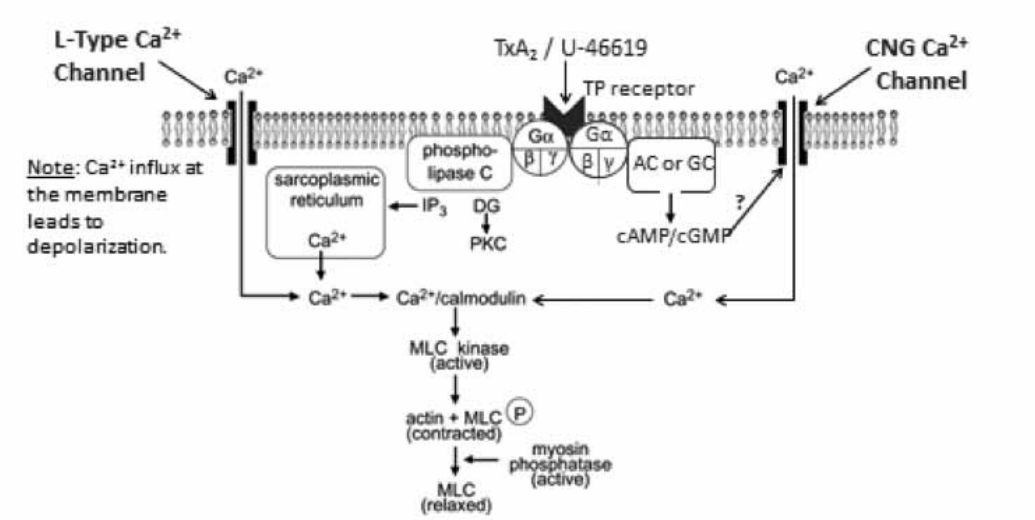
The data obtained from these experiments support the hypothesis that inhibition of L-type and CNG Ca^{2+} channels does, indeed, lead to increased rates of relaxation in U-46619-treated vessels. The mean rate of relaxation for the vehicle (control) treatment group was significantly reduced in comparison to groups receiving nifedipine only, L-cis-diltiazem only, or both inhibitors simultaneously. However, the vessels treated with only one inhibitor—either nifedipine or L-cis-diltiazem—displayed rates of relaxation similar to vessels treated with both inhibitors simultaneously. This result was unexpected, and contrary to the prediction that inhibition of both types of Ca^{2+} channels would lead to significantly greater rates of relaxation than vessels in which only one type of Ca^{2+} channel was inhibited. While inconsistent with original predictions, this observation is equally significant. Thus, it was observed that inhibition of either set of Ca^{2+} channels led to a significant drop in the efficacy of the TxA_2 mechanism for prolonging vascular contraction. We conclude that simultaneous, optimal functioning of both L-type and CNG Ca^{2+} channels is necessary in order to maintain the prolonged contractions characteristic of U-46619-treated vessels.

L-type Ca^{2+} channels have long been associated with the TxA_2/U-46619-induced contraction. These channels are considered to be more abundant in the plasma membranes of vascular smooth muscle cells, and serve as the primary source of Ca^{2+} influx (1, 6). They are also thought to be essential in establishing the initial contraction; though it has been shown that a small

contraction can still be obtained by addition of U-46619 to vessels pre-treated with L-type Ca^{2+} channel inhibitor, nifedipine (6). L-type Ca^{2+} channels have two different gating mechanisms—they are both voltage-dependent and dependent upon allosteric modification (phosphorylation) from protein kinase C (1). When the membrane is sufficiently depolarized, and when the channel is in its phosphorylated state, it opens to the extracellular environment. At this point, L-type channels facilitate the selective influx of Ca^{2+} ions, thereby increasing intracellular Ca^{2+} concentrations.

While the mechanism for L-type channel opening/closing is well known, the function of CNG Ca^{2+} channels remains mostly unexplored. These channels are known to be gated by cyclic nucleotides (such as cAMP or cGMP) in some fashion (12). Because cGMP is often associated with vascular relaxation through the nitric oxide (NO) pathway, it is more likely that cAMP is responsible for mediation of CNG Ca^{2+} channels. Regardless of the pathway involved, it has been demonstrated by this experiment that CNG Ca^{2+} channels are essential to prolonging U-46619-induced vascular contractions, since inhibiting these channels significantly increases the rates of relaxation in aortic vessels.

FIGURE 6.



Shown is a flow chart outlining the proposed mechanism for prolonged vascular contractions induced by TxA_2/U-46619 based on the results of this experiment. Here, the TP receptor is thought to activate another (currently unidentified) G protein, which proceeds to trigger a signaling cascade resulting in the opening of CNG Ca^{2+} channels. Ca^{2+} influx through these channels is thought to serve a variety of mechanisms (as discussed in the text) for prolonging TxA_2-induced vascular contraction (1).

Figure 6 provides a revised schematic of the TxA_2/U-46619 pathway for vascular smooth muscle contraction. It has been modified to reflect the CNG Ca^{2+} channel-dependent mechanism proposed in light of our results. For simplicity, the Rho-kinase pathway has been deleted from this diagram, although it remains a key component of the overall mechanism for prolonged vascular contraction.

The cascade resulting from Gαq protein activation is the same as was proposed originally. In this revised pathway, the TxA_2 receptor (the TP receptor) is coupled to yet another G protein. Though this G protein has not been identified, it may be a Gαs subunit-containing protein. Activation of this G protein is then likely to lead to activation of either guanylate cyclase or adenylate cyclase. The latter is more likely, due to the cGMP mechanisms discussed earlier. At this point, the cyclic nucleotides responsible for regulating the opening of the CNG Ca^{2+} channels are produced. These second messengers lead to further modification of other cellular proteins, which ultimately lead to an allosteric modification of CNG Ca^{2+} channels, promoting the open state.

Once open, the CNG Ca^{2+} channels allow Ca^{2+} influx from the extracellular environment. Ca^{2+} influx through these channels in particular is thought to promote prolonged vascular contraction via three different mechanisms. The most direct effect would be a contribution to the formation of Ca^{2+}/Calmodulin kinases, which lead to cross-bridge cycling and promotion of the contracted state, as discussed earlier. This mechanism alone would explain the persistent (albeit diminished) nature of the contraction following pre-treatment with L-type Ca^{2+} channel inhibitors observed by Liu et al in 1997 (6). CNG Ca^{2+} channels also have the possibility of promoting contraction through two indirect means, both of which involve contributions to the open state of the more traditionally acknowledged L-type Ca^{2+} channels.

The first indirect means involves the voltage-gated property of L-type Ca^{2+} channels. By acting as another rout for Ca^{2+} to enter the cell through the plasma membrane, CNG channels lead to a more depolarized state in the vicinity of the L-type channels. This increases the likelihood of the latter remaining open for longer periods of time. The second strategy for keeping the L-type channels open for prolonged periods involves Ca^{2+} entering through the CNG channels and working with diacyl glycerol to activate protein kinase C. Because this is independent from previously discussed mechanisms for PKC activation, this pathway can be speculated to lead to greater amounts of active PKC in the cell at any given time, thus promoting the phosphorylated, open state of L-type Ca^{2+} channels.

Based on this model, CNG Ca^{2+} channels are able to elicit their effects not only via a direct route, as is the case with L-type Ca^{2+} channels, but also in an indirect manner by promoting the open state in L-type channels. Therefore, we believe that L-type and CNG Ca^{2+} channels function in an interdependent fashion to elicit the prolonged vascular contractions characteristic of TxA_2/U-46619-treated vessels. When L-type Ca^{2+} channels are inhibited by nifedipine, the primary source of extracellular Ca^{2+} has been removed from the equation. This eliminates the cooperative mechanism for prolonging TxA_2-induced vascular contractions, and relaxation ensues. In the absence of functioning L-type channels, the effects of CNG channels are much less significant. On the other hand, when CNG Ca^{2+} channels are inhibited by L-cis-diltiazem, an equally essential component of the system is removed. Here, the cell retains its primary source of Ca^{2+} influx in the form of the L-type channels; however, these channels returned to the closed state much more quickly. This is thought to be analogous to the case of PE or KCl-induced contractions, in which CNG channels likely do not play a role. Thus, early L-type channel closure during CNG channel inhibition is thought to be due to lack of promotion of the open state by increased PKC activity and decreased membrane potential, both of which normally result from the increased Ca^{2+} influx provided by CNG channels.

In each case discussed, the interdependent, cooperative Ca^{2+} channel system for prolonged contraction has been eliminated due to inhibition of one component. Therefore, when both channels are inhibited simultaneously, the increase in rate of relaxation is synonymous to the increase observed when only one channel is inhibited. This is because individual inhibition of either channel has an indirect, inhibitory effect on the other, leaving nothing more to be accomplished by dual or simultaneous inhibition.

LIMITATIONS OF THE METHODS

This investigation has several limitations. It is our assumption that the inhibitors used in these experiments are exerting specific effects on the channels of interest without non-specific effects on other channels or intracellular signaling molecules. This assumption appears to be reasonable, based on previously reported data in the literature (6, 12). We also relied on literature sources

for the doses of the inhibitors used in these experiments. Once again, this seems reasonable based on previous investigations geared toward carefully obtaining dose response data as a means for determining appropriate doses for similar experimental preparations.

SIGNIFICANCE / CLINICAL IMPLICATIONS

This study has clinical significance with respect to disorders in which tissue is damaged as a result of inadequate oxygen and nutrient supply due to decreased blood flow. In the chronic sense, TxA_2-related vascular tension has been identified as a contributor to poor coronary artery circulation underlying angina pectoris (4). Of even greater interest are acute cases of vessel constriction resulting in myocardial infarction (heart attack) or stroke. In these circumstances, blood supply to the tissues in question (either cardiac or neural tissues, both of which have high requirements for oxygen, glucose, etc.) is decreased, resulting in severe tissue damage, and even necrosis (3). This trauma prompts the release of TxA_2, which elicits powerful, prolonged vasoconstrictive effects that further decrease blood supply. Therefore, the presence of TxA_2 in these cases induces a positive feedback cycle in which tissue trauma leads to more vasoconstriction, which leads to more severe tissue trauma, etc. By elucidating the mechanisms responsible for significantly prolonging TxA_2-induced vascular contractions, it is possible that effective treatments for the inhibition of this phenomenon could be developed. Moreover, outlining unique components of these mechanisms could prove especially important for providing treatments capable of specifically targeting TxA_2-induced vascular tension, thereby avoiding negative side effects resulting from interfering with basal systemic vascular tension.

Based upon the results obtained in this experiment, it can be concluded that both L-type and CNG Ca^{2+} channels are necessary, but not sufficient for prolonging TxA_2/U-46619-induced vascular contractions. Furthermore, following the logic and speculations discussed above, it can be concluded that these two types of Ca^{2+} channels are linked together in an interdependent, cooperative mechanism whose optimal functioning is necessary for prolonging the vascular contractions traditionally observed in TxA_2/U-46619-treated vessels.

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